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Review

Intermolecular interactions between natural polysaccharides and silk fibroin protein

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ABSTRACT

Fabricating novel functional and structural materials from natural renewable and degradable materials has attracted much attention. Natural polysaccharides and proteins are the right natural candidates due to their unique structures and properties. The polysaccharide-protein composites or blends were widely investigated, however, there are few systematical studies on the interactions between natural polysaccharides and silk fibroin protein at the molecular level. Among various interactions, hydrogen bonding, electrostatic interactions and covalent bonding play important roles in the structure and properties of the corresponding materials. Therefore, the focus is placed on the three interactions types in this review. A future challenge is to create polysaccharide and protein composites or blends with tailored structure and properties for the wide applications.

Contents

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Nomenclature

AN	Alginate
AFM	Atomic force microscopy
BC	Bacterial cellulose
CMCT	Carboxymethyl chitin
CMCS	Carboxymethyl chitosan
CE	Cellulose
CA	Cellulose acetate
CW	Cellulose whisker
CT	Chitin
CS	Chitosan
DP	Degree of polymerization
DMTA	Dynamic mechanical thermal analysis
ECM	Extra-cellular matrix
FTIR	Fourier transform infrared spectroscopy
T_g	Glass transition temperature
GA	Glutaraldehyde
G (Gly)	Glycine
GAGs	Glycosaminoglycans
GlcA	D-glucuronic acid
GlcNAc	N-acetyl-D-glucosamine
HR	Heparin
HA	Hyaluronic acid
IR	Infrared
IPN	Interpenetrating polymer network
IEP	Isoelectric point
NTU	Nephelometric Turbidity Unit
NMR	Nuclear magnetic resonance
pH_c	pH critical
PBS	Phosphate-buffered saline
SEM	Scanning electron microscope
S (Ser)	Serine
SF	Silk fibroin
TGA	Thermal gravimetric analysis
Y (Tyr)	Tyrosine
V (Val)	Valine
wt	Weight
XRD	X-ray diffraction

1. Introduction

Recently, the preparation of novel functional and structural materials inspired from natural materials has attracted considerable interest (Bhushan & Jung, 2011; Bosia, Buehler, & Pugno, 2010; Deng et al., 2011; Fratzl & Weinkamer, 2007; Gao, Ji, Jager, Arzt, & Fratzl, 2003; Lakes, 1993; Liao, Cui, Zhang, & Feng, 2004; Meyers, Chen, Lin, & Seki, 2008; Munch et al., 2008; Ozin & Oliver, 1995; Pommet et al., 2008; Tan & Saltzman, 2004; Yao, Fang, Wang, & Yu, 2011). Furthermore, increasing problems of energy and developing environmentally friendly functional composites have promoted the usage of renewable resources. Among various types of renewable resources, natural polysaccharides and proteins have shown a wide range of applications in textiles, biodegradable matrices, enzyme immobilization, biomedical area, in forms of fibers, films and scaffolds (Atsumi, Hanai, & Liao, 2008; Buehler, Keten, & Ackbarow, 2008; Dwek, 1996; Kurita, 2001; Li et al., 2009; Ma & Yu, 2004; Rubin, 2008; Yang et al., 2009; Yang & Zhang, 2009). Other than environmental stimuli (such as temperature, humidity, pH value, exposure to alcohol) which influence the structure and properties of composites composed of natural polysaccharides and proteins, intermolecular interactions in the matrices also play a significant role (Dickinson, 1998; Fukui, Feizi, Galustian, Lawson, & Chai, 2002; Oliver, Melton, & Stanley, 2006; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003; Yu, Dean, & Li, 2006).

In the past, however, many review papers concerning intermolecular interactions between natural polysaccharides and proteins focused on the blending solutions (Dickinson, 1998; Tolstoguzov, 1991), while few on other forms like fibers, films and scaffolds (Hardy & Scheibel, 2010). Therefore, we carried out systematic investigations into the intermolecular interactions between natural polysaccharides and silk fibroin protein at the molecular level in their composites or blends in various morphologies in this review.

In order to investigate the intermolecular interactions between natural polysaccharides and silk fibroin protein, a general idea is demonstrated in Fig. 1. Firstly, cellulose, chitin, chitosan, alginate, heparin and hyaluronic acid (or named hyaluronan) are selected as the objects due to their unique properties and wide applications. Secondly, silk fibroin is also considered as the candidate attributed to its detectable conformational transition and wide applications in materials, biotechnological and biomedical science. Thirdly, natural polysaccharides and silk fibroin protein are processed, respectively, until different morphologies of composites or blending solutions are obtained. In the matrix or blending solutions, various silk fibroin structures appear, leading to different properties for diverse applications. The intermolecular interactions between natural polysaccharides and silk fibroin protein may occur when they are mixed, blended or coated. These intermolecular interactions are aiding mediating the structures and properties for applications and the aiding effect sometimes could be more important and necessary than others factors. Therefore, in this review, our work is to highlight effect of such intermolecular interactions between natural polysaccharides and silk fibroin protein on structures and properties.

In general, two types of interactions occur in or between macromolecules, intra-molecular and intermolecular interactions. The former often includes covalent bonding and intra-molecular hydrogen bonding, while the latter includes intermolecular hydrogen bonding, electrostatic interactions, intermolecular covalent bonding and hydrophobic interactions (Dickinson, 1998).

Hydrophobic interactions, as an important kind of intermolecular interactions, often occur, respectively, in natural polysaccharides (Desbrières, Hirrien, & Rinaudo, 1998) and silk fibroin protein (Wang et al., 2007), rather than bridging between their heterogeneous molecules. Therefore, hydrophobic interactions are not to be discussed in detail in this review and the focus is placed on hydrogen bonding, electrostatic interactions and covalent bonding.

2. Structures of silk fibroin protein and polysaccharides

In order to clarify intermolecular interactions between silk fibroin protein and natural polysaccharides, their structures are first discussed.

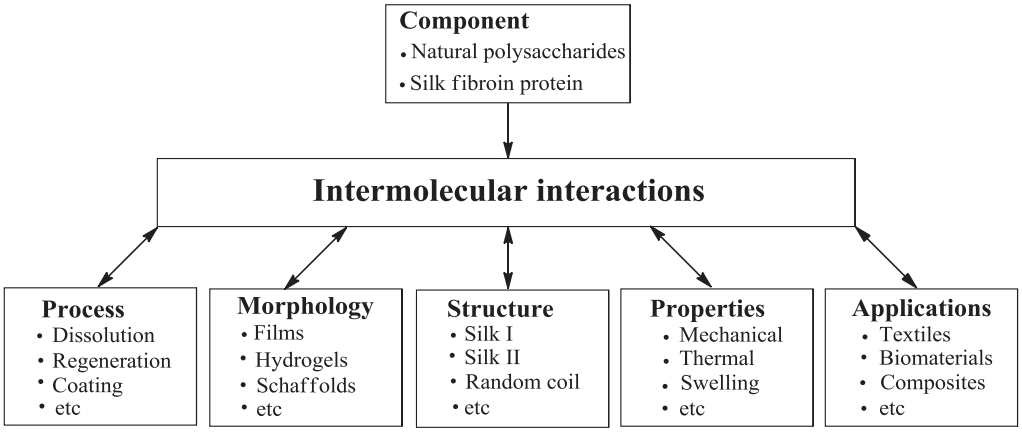

Fig. 1. Relationship between intermolecular interactions and other factors.

2.1. Structures of Bombyx mori silk

Silks are produced by silkworms, spiders, scorpions, mites and flies, and are different in component, structure and properties according to specific source (Altman et al., 2003; Vepari & Kaplan, 2007). At present, the most studied silks in textiles, biotechnological and biomedical fields are domesticated silkworm silks, identified as *Bombyx mori*. As shown in Fig. 2, *Bombyx mori* silk presents unique hierarchical features, i.e. the dominant part of silk fibroin protein, is surrounded by another type of silk protein, which is sericin. Sericin coats the silk fibroin and acts as an adhesive. The two fibroin filaments join together and are formed in one *Bombyx mori* silk filament (Zhao & Asakura, 2001).

Compared with other proteins, silk fibroin protein is characterized by an Ala-Gly-X primary sequence, leading to regular conformations at its primary level (Altman et al., 2003; Vepari & Kaplan, 2007). Recently, the sequence of the Bombyx silk gene has been completed (Ha, Gracz, Tonelli, & Hudson, 2005; Zhou et al., 2000). In detail, the repeating region comprises various units, including highly repetitive GAGAGS hexamer and less repetitive GAGAGY (the less organized sequence) or/and AGVGYGAG motifs (Ha et al., 2005; Zhou et al., 2000). It can be seen from Fig. 3 that strong intermolecular hydrogen bonding is formed which benefits the generation of a thermodynamically stable structure and the indissolubility in some solvents. On the other hand, the less ordered regions contain a lot of amino acid residues (Ha et al., 2005; Zhou et al., 2000; Valluzzi, Gido, Muller, & Kaplan, 1999). XRD analyses of the crystalline part of silk proteins indicate that the polypeptides arranged in fully extended forms (Mondal, Trivedy, & Kumar, 2007).

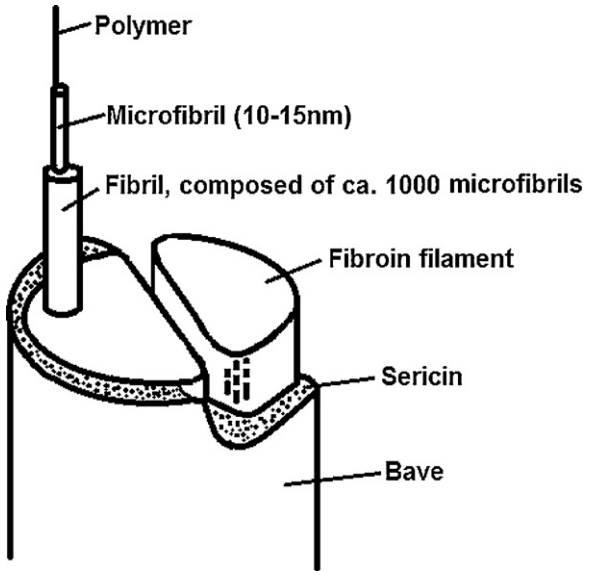

Fig. 2. Hierarchical features of *Bombyx* mori silk.

2.2. Structures of natural polysaccharides

2.2.1. Cellulose

It is generally accepted that cellulose is the most abundant organic polymer in the world and is regarded as a renewable resource (Klemm, Heublein, Fink, & Bohn, 2005). Cellulose is widely distributed in plant fibers, such as wood and cotton, and it can not only be used in building and textile materials, but in liquid crystalline and biocompatible materials (Klemm et al., 2005; Kobayashi, Sakamoto, & Kimura, 2001). As one kind of polysaccharides, cellulose has different crystal patterns, among which cellulose I and II are studied most. Cellulose I is the main pattern while cellulose II is the mercerized or recrystallized pattern of cellulose I. Analogous to chitin, cellulose is a linear-chain polymer with a β (1→4) structure. In detail, Fig. 4 shows the molecular structure of cellulose, indicating that cellulose is produced from many β-D-glucopyranose molecules that are covalently linked through acetal reactions between the equatorial hydroxy group of C4 and the C1 carbon atom (β-1,4-glucan). In addition, the OH groups of β-1,4-glucan cellulose are formed at the positions of C2, C3 and C6 (Klemm

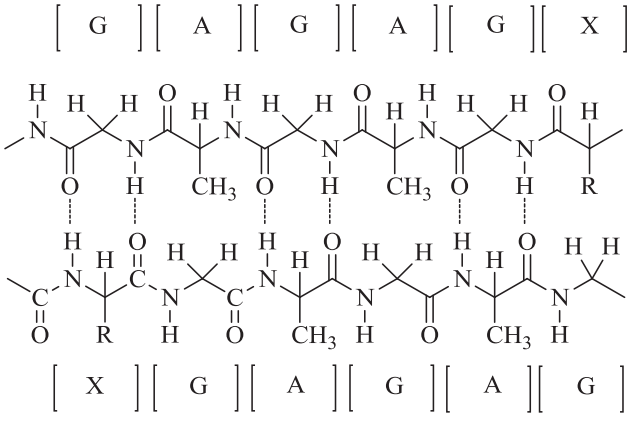

Fig. 3. Molecular structures of silk fibroin.

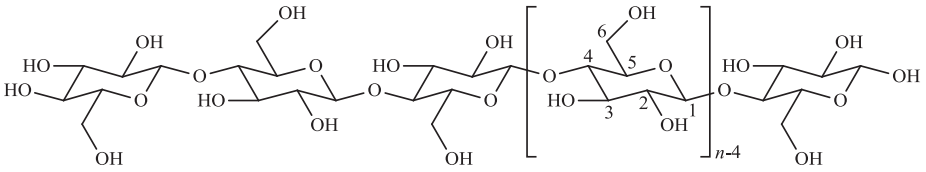

Fig. 4. Molecular structure of cellulose.

et al., 2005). In the three dimensional network of the cellulose, hydrogen bondings play a very important role and can be responsible for some unique properties, such as the difficulty in dissolving in common solvents.

2.2.2. Chitin and chitosan

Chitin, the second most abundant natural muco-polysaccharide, is synthesized in an enormous number of living organisms (Rinaudo, 2006). Chitin consists of (β-(1→4)-N-acetyl-D-glucosamine), which can be degraded by chitinase. It can be considered that cellulose has its OH group at position C2 replaced by an acetamido group. Similar to cellulose, chitin can function naturally as a structural polysaccharide. On the other hand, chitosan, another important polysaccharide, is the partial N-deacetylated derivative of chitin (Kumar, 2000). Fig. 5 shows the molecular structures of chitin and chitosan, which are slightly different from that of cellulose illustrated in Fig. 4 (Rinaudo, 2006). However, quite different from cellulose, chitosan can be solved in dilute acetic acid due to its protonation effect, which promotes the application of chitosan in many fields (Kumar, 2000; Rinaudo, 2006).

2.2.3. Alginate

Alginates are regarded as a kind of linear unbranched polysaccharides, which feature the fraction and sequence of the two monomers, α-L-guluronic acid (G) and β-D-mannuronic acid (M) (Fig. 6) (Draget, SkjakBraek, & Smidsrod, 1997; Gombotz & Wee, 1998; Rowley, Madlambayan, & Mooney, 1999). The homo-polymeric regions of β-D-mannuronic acid blocks and α-L-guluronic acid blocks are interdispersed with parts of alternating structure. However, alginates vary in amount and sequential distribution according to the source of the alginate (Draget et al., 1997; Gombotz & Wee, 1998; MacGregor & Greenwood, 1980; Rowley et al., 1999). In general, alginates can be applied in biotechnology, food and beverage industry, pharmacy industry due to their unique properties.

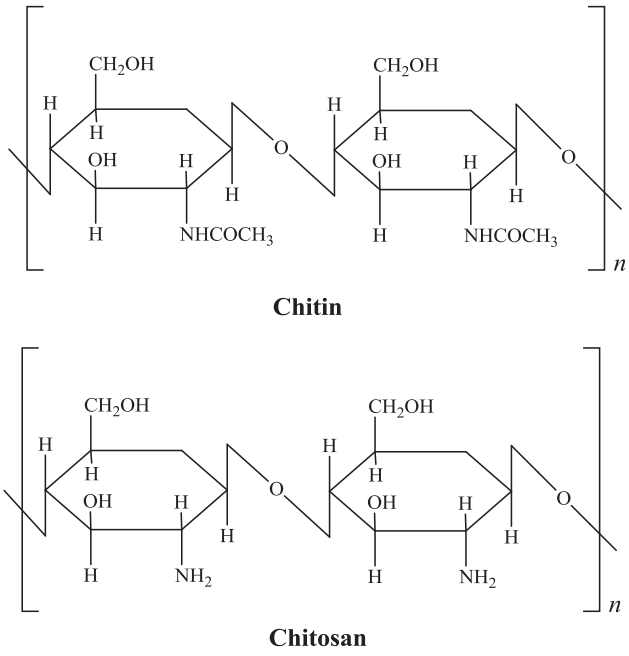

Fig. 5. Molecular structures of chitin and chitosan.

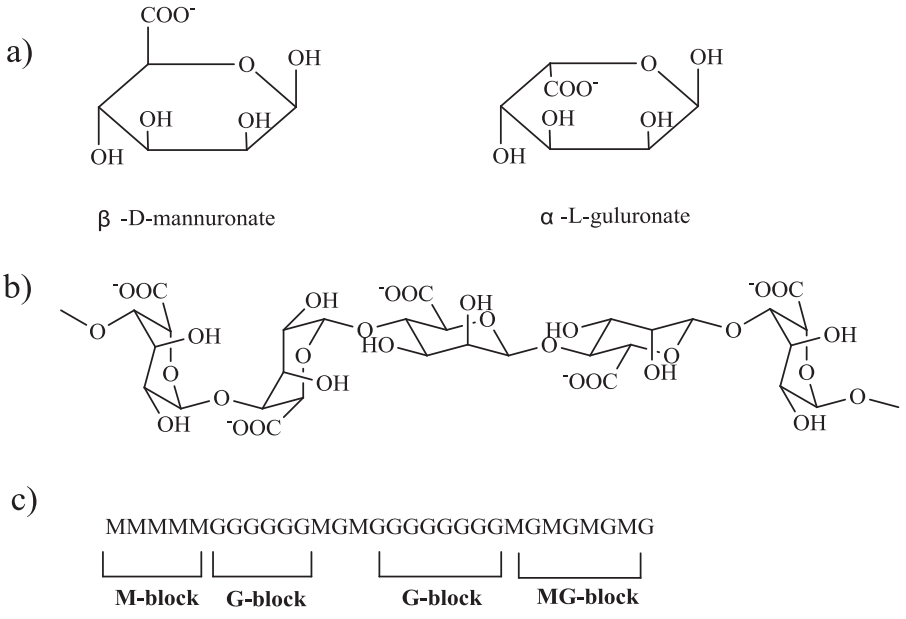

Fig. 6. Molecular structure of alginate.

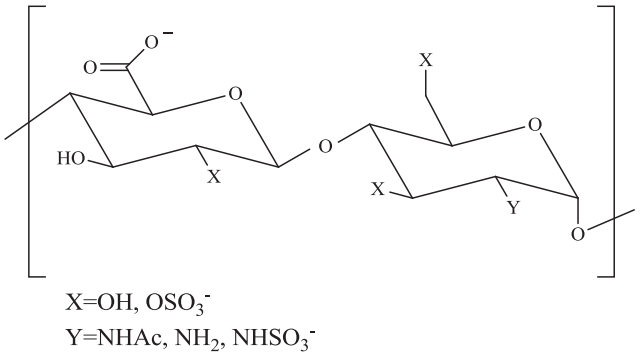

Fig. 7. Molecular structures of heparin and heparan sulfate.

2.2.4. Heparin

Heparin and heparan sulfate are common polysaccharides in mammalian tissues which are also important glycosaminoglycans (GAGs). The glycosaminoglycans are composed of repeated disaccharide units (Fig. 7) and are fully charged polyanions containing carboxylate and amino groups (Boddohi & Kipper, 2010; Lapcik, De Smedt, Demeester, & Chabrecek, 1998). Heparin and heparan sulfate have similar molecular structures, and they consist of more than ten different monosaccharide building blocks (Lindahl, Kusche, Lidholt, & Oscarsson, 1989; Salmivirta, Lidholt, & Lindahl, 1996). Due to the unique characteristics, heparin and heparan sulfate can form electrostatic interactions with different proteins.

2.2.5. Hyaluronic acid

As another important glycosaminoglycan, hyaluronic acid (or named hyaluronan) is generally found in the extra-cellular matrix or synovial fluids, and have a relatively high molecular weight (Burdick & Prestwich, 2011; Lapcik et al., 1998; Rapport, Weissmann, Linker, & Meyer, 1951). It is composed of a repeating disaccharide that consists of N-acetyl-D-glucosamine (GlcNAc) and D-glucuronic acid (GlcA) bridged by a β-1,4-glycosidic bond (Fig. 8) (Burdick & Prestwich, 2011). Similar to alginates, hyaluronic acid is also an unbranched polymer. In addition, hyaluronic acid is a highly hydrated polyanionic polymer, with a molecular weight from 100 kDa in serum to 8000 kDa in the vitreous body (Burdick & Prestwich, 2011).

3. Structural characterization of interactions between natural polysaccharides and silk fibroin protein

Although the XRD technique is an effective method to elucidate the crystalline structure, it is less effective for the determination of the interactions between fibrous proteins and polysaccharides. It is generally accepted that vibration spectra, including IR (Asakura, Kuzuhara, Tabeta, & Saito, 1985; Barth & Zscherp, 2002; Hu, Kaplan, & Cebe, 2006; Jackson & Mantsch, 1995; Pelton & McLean, 2000; Shang et al., 2009) and Raman (Monti, Freddi, Bertoluzza, Kasai, & Tsukada, 1998; Rousseau, Lefevre, Beaulieu, Asakura, & Pezolet, 2004) spectroscopy, together with nuclear magnetic resonance (NMR) (Ducel, Pouliquen, Richard, & Boury, 2008; Zhao & Asakura, 2001; Zhou et al., 2004) spectroscopy, can provide sensitive means for characterizing the secondary structural elements of the protein, such as α-helical, β-sheet and random coil conformations. Therefore, such instrumental measurements are an effective tool for assessing the protein–macromolecule interactions and can contribute to the target of identification and selection. IR spectra come from the absorption of energy by vibrating chemical bonds, while Raman spectra result from the same kinds of transitions with different selection rules (Gombotz & Wee, 1998). Although Raman and NMR can reflect the conformation transition of proteins and polysaccharides as well, the IR spectrum is selected in this review.

3.1. Introduction

According to the relevant literature, the conformation transition of silk fibroin can be easily observed due to the metastable state of the α-helices and the stable β-sheet in silk fibroin (Chen et al., 2001; Fossey, Nemethy, Gibson, & Scheraga, 1991; Shang, Zhu, & Fan, 2011). Similarly, when silk fibroin is blended with polymers containing hydroxyl, the cross-linking of silk fibroin and such polymers may benefit the conformation transition of silk fibroin. It is generally accepted that, if there are interactions between proteins and polysaccharides, the conformation of proteins would change, which is detectable by IR analysis. Therefore, in this review, the focus of the structural characterization of interactions between the fibrous protein and polysaccharides is on the silk fibroin–polysaccharides interactions.

3.2. Spectrum analysis

To the best of our knowledge, nine normal patterns for the amide bands of proteins are named A, B and I–VII in order of decreasing frequency (Pelton & McLean, 2000). Among all the amide bands of proteins, amide bands I, II and III are generally selected to differentiate protein structures. Table 1 summarizes the IR spectra of these three amide bands.

The amide I band of proteins is conformation sensitive, together with little chance of being overlapped by other bands. Kaplan et al. summarized the wave-number ranges corresponding to vibrational bands in *Bombyx mori* silk within the amide I region of the spectrum (Table 2) (Hu et al., 2006).

Sometimes, in order to simplify the evaluation process of the conformation transition in silk proteins, α-helical and random coil structures could both be ascribed to Silk I structure, while β-sheets to the Silk II structure. Table 3 lists the amide bands of the IR spectra of silk fibroin with different structures.

According to the discussion above, Table 4 lists the structural changes due to interactions between the fibrous protein and

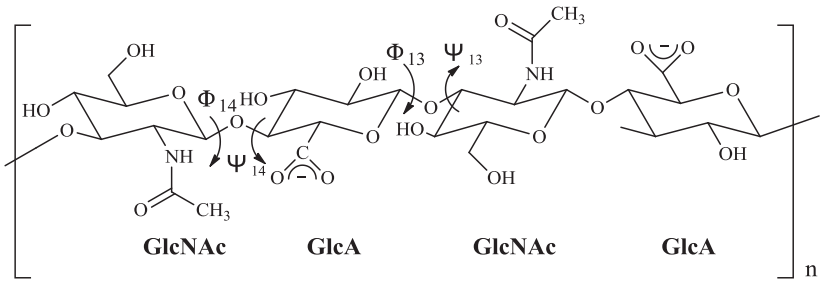

Fig. 8. Molecular structure of hyaluronan.

Table 1
Comparison of amide bands (I, II and III) of IR spectroscopy (Pelton & McLean, 2000).

Band	Comments
Amide I	Proteins known to adopt an α-helical conformation have strong amide I bands between 1650 and 1655 cm^{-1}. The hydrogen-bonding strengths in β-sheets are more variable due to their flexibility and tendency to twist. A strong band between 1612 and 1640 cm^{-1} and a weaker band about 1685 cm^{-1} are commonly observed for β-sheets, although weak bands at somewhat lower frequencies (1665–1670 cm^{-1}) have also been observed.
Amide II	A strong amide II band is observed at 1540–1550 cm^{-1} and a weaker shoulder at 1510–1525 cm^{-1}. Peptides and proteins with an antiparallel β-sheet structure have strong amide II bands between 1510 and 1530 cm^{-1}; a parallel β-sheet structure is found at somewhat higher frequencies (1530–1550 cm^{-1}).
Amide III	The amide III band is normally quite weak and occurs in a region of mixed vibrations (CH bending, tyrosine and phenylalanine ring vibrations) that are not readily correlated to protein secondary structure.

Table 2
Vibrational band assignments in the amide I region for *Bombyx* mori silk fibroin (Hu et al., 2006).

Wavenumber range (cm^{-1})	Assignment
1605–1615	(Tyr) Side chains/aggregated strands
1616–1621	Aggregate beta-strand/beta-sheets (weak)[a]
1622–1627	Beta-sheets (strong)[a]
1628–1637	Beta-sheets (strong)[b]
1638–1646	Random coils/extended chains
1647–1655	Random coils
1656–1662	Alpha-helices
1663–1670	Turns
1671–1685	Turns
1686–1696	Turns
1697–1703	Beta-sheets (weak)[a]

[a] Intermolecular beta-sheets.
[b] Intramolecular beta-sheets.

polysaccharides which are determined by IR analysis (Chen, Li, Shao, Zhong, & Yu, 1999; Chen, Li, & Yu, 1997; Chen, Li, Zhong, Lu, & Yu, 1997; Du, Zhu, Chen, & Xu, 2006; Ducel et al., 2008; Freddi, Romano, Massafra, & Tsukada, 1995; He, Wang, Cui, Gao, & Wang, 2010; Kang, Lee, Ki, Nahm, & Park, 2004; Kundu, Patra, & Kundu, 2008; Kuzmina, Sashina, Novoselov, & Zaborski, 2009; Kweon, Ha, Um, & Park, 2001; Liang & Hirabayashi, 1992; Lin, Yao, Chen, & Wang, 2008; Malay, Bayraktar, & Batigun, 2007; Niamsa, Srisuwan, Baimark, Phinyocheep, & Kittipoom, 2009; Noishiki, Nishiyama, Wada, Kuga, & Magoshi, 2002; Sampaio, Taddei, Monti, Buchert, & Freddi, 2005; Sashina, Janowska, Zaborski, & Vnuchkin, 2007; Park, Lee, Ha, & Park, 1999; She et al., 2008; She, Zhang, Jin, Feng, & Xu, 2008; Wang, Zhang, Wang, & Dong, 2011; Wongpanit, Tabata, & Rujiravanit, 2007;Yang, Zhang, Cao, & Liu, 2002; Yang, Zhang, & Liu, 2000; Zhou et al., 2004). It can be seen that the hydrogen bonding formation is usually accompanied by a peak shift in the amide bands of the silk fibroin, and by the conformation transition of the silk fibroin as well.

Table 3
Comparison of amide bands of IR spectroscopy for silk fibroin with different structures (Asakura et al., 1985).

Structures	Silk I/random coil	Silk II
Amide I	1660	1630
Amide II	1550[a]	1530
Amide III	1235	1265
Amide V	650	700

[a] Authors added.

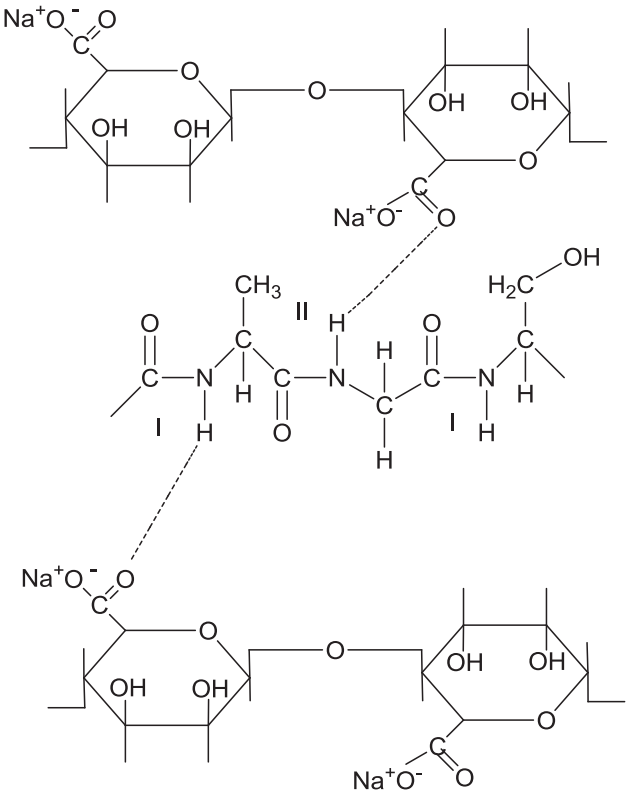

Fig. 9. Hydrogen bonds between fibroin and sodium alginate.

4. Forming of the interactions

In order to better understand the effect of intermolecular interactions between natural polysaccharides and silk fibroin protein, their forming processes are introduced in this part. As discussed above, the focus is placed on hydrogen bonding, electrostatic interactions and covalent bonding.

4.1. Hydrogen bonding

Liang and Hirabayashi (1992) applied the solution blending and film cast method to obtain silk fibroin-alginate blending films. They found that the peaks in the amide I and II IR spectra shifted to lower wave-numbers and the weak and broad, unoriented crystalline peaks of pure silk fibroin converted to characteristic crystalline diffraction peaks, suggesting that the intermolecular interactions between silk fibroin and alginate promoted the formation of the β-sheet conformation of silk fibroin. Therefore, they stated hydrogen bonding was the intermolecular interaction between alginate and silk, as shown in Fig. 9. This work may be the pioneering investigation of polysaccharide–silk fibroin protein intermolecular interactions.

Inspired by the silk fibroin–alginate interactions, Chen, Li, and Yu (1997) carried out the silk fibroin-chitosan blending experiments and structural analyses before they proved that the silk fibroin formed the β-sheet structure in the blending films. In particular, when the ratio of silk fibroin to chitosan was 1:9, most of silk fibroin exhibited the β-sheet structure, indicating that hydrogen bonding could be formed between the silk fibroin and chitosan in the blending films. They (Chen, Li, & Yu, 1997) further stated that the silk fibroin could utilize the rigid chitosan molecules to extend silk molecules, resulting in the forming of β-sheet conformation caused by strong hydrogen bonding between silk fibroin

Table 4
Structural change due to interactions between silk fibroin and polysaccharides by IR analysis.

Component	Morphologies	Type of IA	Vibrational Band	Comments	Ref.
Silk fibroin-cellulose	Film	Mainly hydrogen bonding	Peaks change	β-sheet and random coil structures co-exist	Freddi et al. (1995)
Silk fibroin-cellulose	Film	Hydrogen bonding	Peaks in amide A and I shifted to lower wavenumber	Partial random coil conformation of silk fibroin converted into β-sheet conformation	Yang et al. (2000)
Silk fibroin-cellulose	Film	Hydrogen bonding	Peaks broadened in Amide A Peaks in amide I shifted to lower wavenumber	Partial random coil conformation of silk fibroin converted into β-sheet conformation	Yang et al. (2002)
Silk fibroin-cellulose whisker	Film	Hydrogen bonding[a]	Peaks in amide I and II shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Noishiki et al. (2002)
Silk fibroin-cellulose	Film	Hydrogen bonding	Peaks in amide A, I and II shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Kundu et al. (2008)
Silk fibroin-chitosan	Film	Hydrogen bonding	Peaks in amide I and II shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Chen, Li, and Yu (1997)
Silk fibroin-chitosan	Film	Hydrogen bonding[a]	Peaks in amide I shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Park et al. (1999)
Silk fibroin-chitosan	Film	Hydrogen bonding	Peaks in amide I shifted to lower wavenumber and in amide III shifted to higher wavenumber	NH groups of SF and the C=O and NH_2 groups of CS participated intermolecular interaction between silk fibroin and CT	Du, Zhu, Chen, and Xu (2006)
Silk fibroin-chitosan	Film	Hydrogen bonding[a]	Spectral shift of the amide I, II and III	Nil	Wongpanit et al. (2007)
Silk fibroin-chitosan	Film	Hydrogen bonding	Peaks in amide I and II shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Niamsa et al. (2009)
Silk fibroin-chitosan	Film	Hydrogen bonding[a]	Peaks in amide I shifted to lower wavenumber and in amide III shifted to higher wavenumber	Formation of β-sheet conformation of silk fibroin	He et al. (2010)
Silk fibroin-chitosan	Gel	Hydrogen bonding	Peaks in amide I shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Chen, Li, Zhong et al. (1997)
Silk fibroin-chitosan	Scaffold	Hydrogen bonding	Peaks in amide III shifted to higher wavenumber	Formation of β-sheet conformation of silk fibroin	She et al. (2008), She, Zhang et al. (2008)
Silk fibroin-chitosan	Scaffold	Hydrogen bonding	Peaks in amide I and II shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	She, Zhang et al. (2008)
Silk fibroin-alginate	Film	Hydrogen bonding	Bands in amide I and amide II of random coil disappeared	Formation of β-sheet conformation of silk fibroin	Liang and Hirabayashi (1992)
Silk fibroin-cellulose	Cellulose fiber with SF powder	Covalent bonding	New peak appeared	CN bond formed from C=O of CE fiber and NH_2 of silk fibroin	Sampaio et al. (2005)
Silk fibroin-heparin	Film	Covalent bonding	Peaks in amide I and II shifted to lower wavenumber	Formation of β-sheet conformation of silk fibroin	Wang, Zhang, Wang, and Dong (2011)

[a] Authors added.

and chitosan, and they correspondingly proposed the 'Polymer-induced conformation transition' mechanism. According to their mechanism, the conformation transition of fibroin protein can be caused by polymer blending, similar to the effect of alcohols and heat treatment (Drummy, Phillips, Stone, Farmer, & Naik, 2005; Tsukada et al., 1994), which is very important for obtaining natural silk fibroin-based materials, and could be applied to the preparation and modification of other protein-based materials.

4.2. Electrostatic interactions

In general, besides the hydrogen bonding, electrostatic interactions also occur in the polymer matrix (Hattori, Hallberg, & Dubin, 2000; Xu et al., 2004). Malay et al. (2007) studied silk fibroin-hyaluronic acid blending solutions and observed their complex coacervation behavior. They found that when pH was between the pKa value of hyaluronic acid (pH 2.5) and the iso-electric point (IEP) of silk fibroin (pH 3.9), the positively charged silk fibroin and the negatively charged hyaluronic acid could form an electrostatic interaction, resulting in the formation of coacervate.

4.3. Covalent bonding

It is generally accepted that the covalent bonding is much stronger than either hydrogen bonds or electrostatic interactions, and can stabilize the molecules. The covalent bonding can be roughly classified into two kinds, the one directly formed by proteins and polysaccharides, referred to as 'without cross-linkers', and the one bridged by cross-linkers, referred to as 'with cross-linkers'.

4.3.1. Covalent bonding without cross-linkers

Kang et al. (2004) prepared the silk fibroin-chitosan conjugates via covalent bonds, and found that the tyrosine residues in the silk fibroin could be completely changed to *o*-quinone residues by the tyrosinase. The NH_2 groups of the chitosan and the N-terminal NH_2 groups in the silk fibroin could react with the enzymatically generated *o*-quinone residues in the silk fibroin. A reaction between the COOH group of quinone and the NH_2 group could form a Schiff base. Therefore, they proposed a possible procedure of the enzymatic and non-enzymatic reactions between chitosan and silk fibroin. Sampaio et al. (2005) applied the graft technique to obtain a covalently bonded silk fibroin-chitosan matrix. They also stated that the

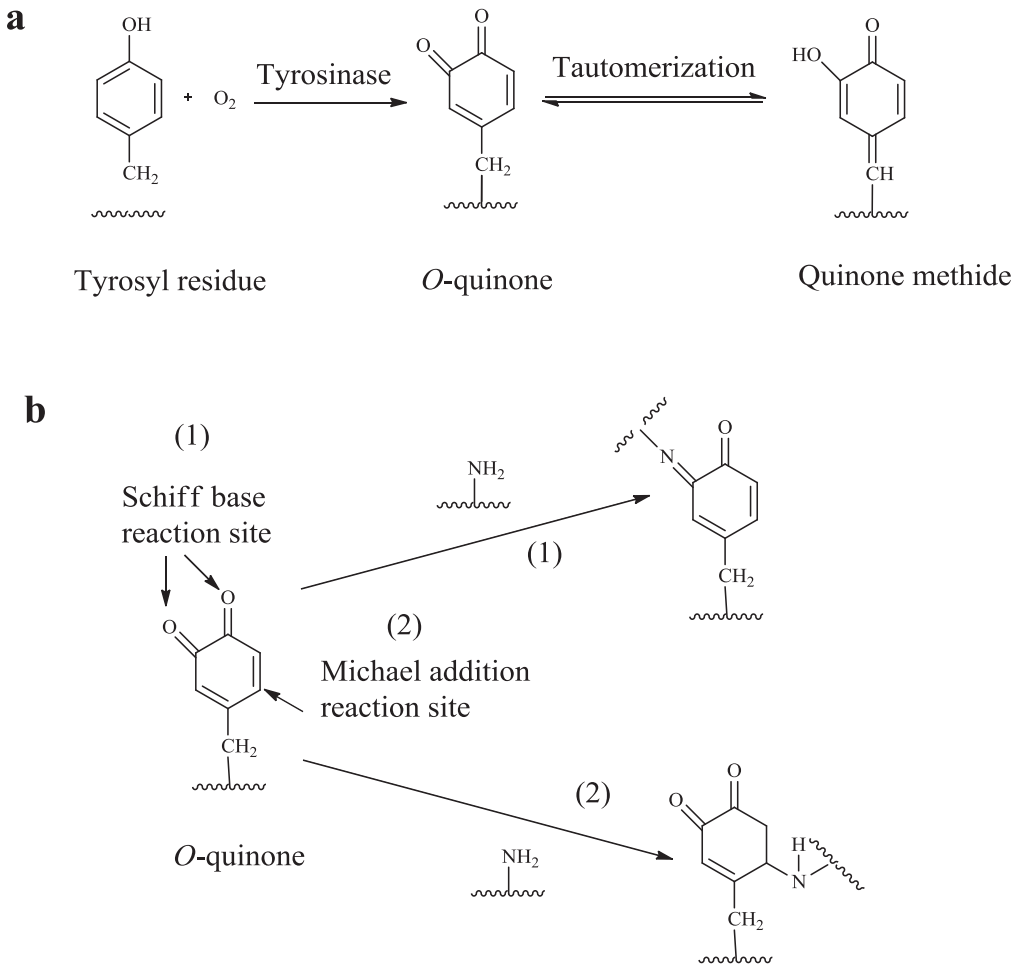

Fig. 10. Oxidization of Tyr residue by tyrosinase (a) and covalent bond forming between quinones and free amine groups (b).

Tyr residue could be oxidized to *o*-quinone with the aid of tyrosinase as catalyst, after which the *o*-quinone could be converted to quinone methide by tautomerization (Fig. 10 (a)). Under favorable conditions, the quinones could be bonded to free amine groups via Schiff-base and/or Michael reaction procedure as shown in Fig. 10 (b). Besides silk fibroin and chitosan, silk fibroin and cellulose can also be covalently bonded. Lin et al. (2008) prepared the silk fibroin modified cotton fiber, via covalent bonding. They pointed out that the covalent bond formation could occur in three steps: the first step is the oxidization of cotton fiber by sodium periodate, the second one is the treating of oxidized cotton fibers by silk fibroin and the third is the cotton fibers covalently bonded silk fibroin through the interactions between active aldehyde group of the former and free amino groups of the latter, resulting in improved physical and mechanical properties of cotton fibers.

4.3.2. Covalent bonding with cross-linkers

At present, the most widely used cross-linkers for proteins and polysaccharides are small glyoxal (Wang & Stegemann, 2011) and glutaraldehyde (Ma et al., 2003), epoxy resin and PEG400 dimethacrylate (Ferrero, Periolatto, Burelli, & Carletto, 2010), together with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (Park, Park, Kim, Song, & Suh, 2002). Besides the common chemical cross-linkers, natural cross-linkers, such as genipin (Butler, Ng, & Pudney, 2003; Datta, Dhara, & Chatterjee, 2012; Silva et al., 2008), also have attracted much attention. Genipin, as a natural cross-linker, can also bridge amino groups between the polymer chains of proteins and chitosan. Genipin is extracted from the fruits of the plant *Gardenia jasminoides Ellis* or obtained from geniposide, which is a component of traditional Chinese medicine (Silva et al., 2008). Silva et al. (2008) added genipin into the silk fibroin-chitosan blending solution to initiate the cross-linking between silk fibroin and chitosan prior to obtaining the silk fibroin-chitosan sponges (Fig. 11). In order to study the reaction pathway how genipin bridged biopolymers with primary amine groups, Butler et al. (2003) carried out instrumental analyses and stated the two reactions which could lead to the cross-linking of polymers containing primary amine groups through genipin.

5. Influence of intermolecular interactions between natural polysaccharides and silk fibroin protein

The structural transitions caused by interactions between natural polysaccharides and silk fibroin proteins are often accompanied with the change of the properties of the polymer matrix. Therefore, Table 5 lists the changes in properties as the result of the interactions between the fibrous proteins and polysaccharides, as well as the corresponding measurement methods.

5.1. Influence of interactions on crease resistance

As a traditional textile dressing, cotton fabrics are comfortable and popular due to their good physical properties (Lin et al., 2008). However, their crease properties cause the wearing

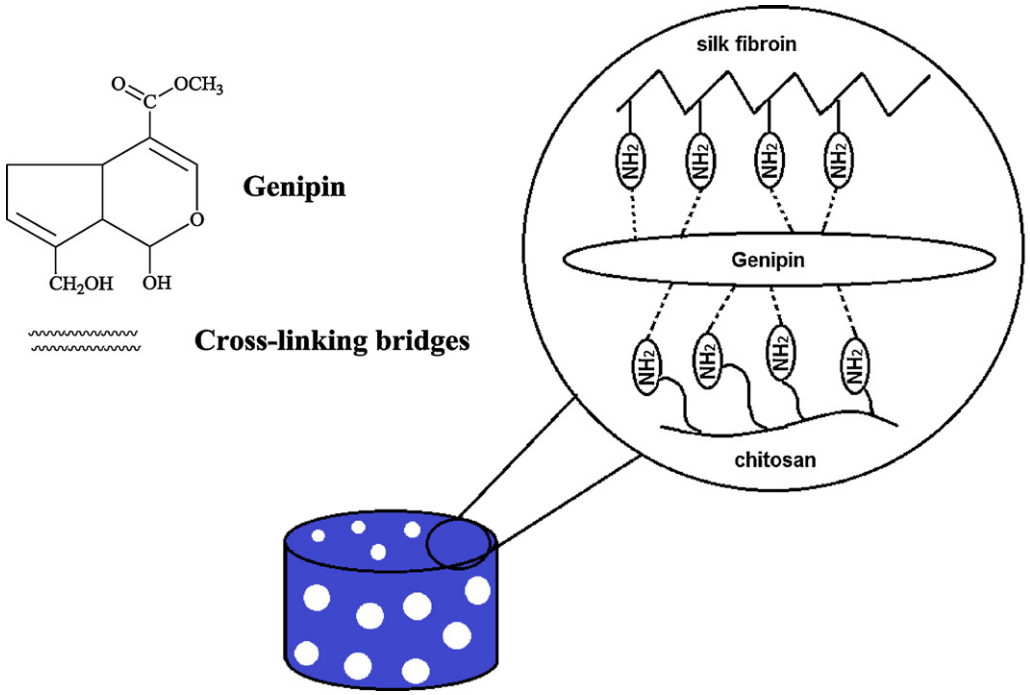

Fig. 11. Schematic representation of the silk fibroin/chitosan hydrogels crosslinked by genipin.

problems. To overcome the wearing drawback of cotton fabrics, Lin et al. (2008) firstly prepared silk fibroin powder by the dissolution–dialysis–lyophilization strategy. Then they obtained oxidized cotton fabrics with sodium periodate treatment. After that they coated the cotton fabrics with silk fibroin solutions and cured the cotton fabrics at 170 °C for a short time. In this way the values of wrinkle recovery angles of the samples increased, indicating that the anti-crease properties of the oxidized cotton fabric coated by silk fibroin were improved.

5.2. Influence of interactions on mechanical properties

Mechanical properties of the blending films are important in many applications. Park et al. (1999) found that the tensile strength of the silk fibroin-chitosan blending membranes depended on chitosan content, and the tensile strength of the blending membranes showed a higher value than that of the pure polymer, perhaps due to the conformation transition and increased β-sheet crystalline of the silk fibroin that led to increased tensile properties. Similar to the results from Park's group, Noishiki et al. (2002) found that the silk fibroin protein–microcrystalline cellulose composite film with 70–80% cellulose content was much stronger than individual components, indicating that the tensile breaking strength of the film anomalously depended on the blending ratio.

5.3. Influence of interactions on thermal properties

Kuzmina et al. (2009) blended cellulose with silk fibroin protein. They used a Setaram instrument to carry out thermal gravimetric analysis (TGA) for cellulose-silk fibroin blends. The thermo-stability of the films from cellulose-fibroin blends was higher than that of pure silk membrane, while lower than that of pure cellulose membrane. Shang et al. (2011) also tested the cellulose-silk blending films with different ratios. The results suggested that the sample with the ratio of cellulose to silk 75:25 had the highest temperature of maximum mass loss rate, caused by the strongest intermolecular interactions in this ratio.

5.4. Influence of interactions on miscibility

Miscibility is also a very significant factor in evaluating the properties of the materials. The common criteria for the compatibility in a polymer blending system are the appearance of a single glass transition, due to the interactions between two different polymers. Park et al. (1999) applied DMTA measurements to investigate the interactions in silk fibroin-chitosan blends. Their work showed an increased temperature of relaxation peak of fibroin protein when it was blended with chitosan, which indicated there were strong interactions between fibroin protein and chitosan. Their work showed that all the silk fibroin-chitosan blends had a single glass transition via dynamic mechanical properties, indicating that silk fibroin-chitosan matrix had good miscibility at the molecular level. In contrast to the previous approaches, Kweon et al. (2001) applied the scanning electron microscope (SEM) to evaluate the miscibility of the silk fibroin-chitosan blends. They found that the silk fibroin-chitosan blending system did not show phase separation morphology, perhaps due to the intermolecular hydrogen bonding between fibroin and chitosan. Recently, Shang et al. (2011) applied atomic force microscopy (AFM) for the morphological observation and the results showed that surface morphology of the blending films depended on cellulose content greatly (Fig. 12).

5.5. Influence of interactions on water stability

In general, a poor biodegradability of the silk fibroin protein is mainly due to its high β-structure content and hydrophobic nature. Wongpanit et al. (2007) found that, upon blending the silk fibroin with carboxy-methyl chitin (CMC), the miscibility of the two polymers appeared only in their amorphous region and that the β-structure of fibroin protein, induced by alcohol treatment, was not affected by carboxy-methyl chitin. Furthermore, a strong intermolecular interaction between fibroin protein and carboxy-methyl chitin caused a good resistance of the blending films from dissolving in a PBS solution, which was different from dissolved glutaraldehyde cross-linked carboxy-methyl chitin films in PBS solution.

Table 5
Properties change due to interactions between silk fibroin and polysaccharides and corresponding measurement methods.

Component	Morphologies	Measurement methods and apparatus	Properties change	Ref.
Silk fibroin-cellulose	Film	Floatation method with xylene-carbon tetrachloride solutions	Density change	Freddi et al. (1995)
Silk fibroin-cellulose	Film	Dynamic mechanical thermal analysis (DMTA) on visco-elastic spectrometer	Miscibility change	Yang et al. (2000)
Silk fibroin-cellulose	Film	Weight retention method DMTA on visco-elastic spectrometer Differential scanning calorimetry (DSC) measurements with Thermomechanical Analyzer	Less SF film lost Miscibility change Higher decomposition temperature	Yang et al. (2002)
Silk fibroin-cellulose	Film	Tensile test with Tensilon/UTM-III-100	Greater tensile strengths	Noishiki et al. (2002)
Silk fibroin-cellulose	Film	Thermogravimetric analysis (TGA) with Setaram instrument	More residues	Sashina et al. (2007)
Silk fibroin-cellulose	Film	Weight retention method[a]	Less film lost	Kundu et al. (2008)
Silk fibroin-cellulose	Film	Viscosity measurements by Reologica Rheometer TGA with Setaram instrument	Viscosity change Slowest thermal destruction of films	Kuzmina et al. (2009)
Silk fibroin-chitosan	Film	DMTA with DMTA MK III Floatation method with carbon tetrachloride/heptane mixture Mechanical tests with Instron 4201	Good miscibility Higher density value than that of a pure polymer Higher tensile strength value than that of a pure polymer	Park et al. (1999)
Silk fibroin-chitosan	Film	Immersed method with ethanol–water mixture as separation model	Separation factor increased and the flux decreased	Chen et al. (1999)
Silk fibroin-chitosan	Film	Morphology observation of the fractured surface with scanning electron microscope (SEM)	No microscopic phase separation morphology	Kweon et al. (2001)
Silk fibroin-chitosan	Film	Immersed method with deionized water	Higher swelling ratio value than that of a pure polymer	Du, Zhu, Chen, and Xu (2006)
Silk fibroin-carboxymethyl chitin	Film	Incubation method in phosphate-buffered saline (PBS)	Good resistance	Wongpanit et al. (2007)
Silk fibroin-chitosan	Film	Moisture equilibrium method Immersed method with water	Lower moisture absorption Lower swelling capacity	He et al. (2010)
Silk fibroin-chitosan	Gel	Immersed method with Britton-Robinson general buffer solutions	Swelling ratio increased in acidic medium	Chen, Li, Zhong et al. (1997)
Silk fibroin-hyaluronic acid	Film	Turbidimetric titrations with Naked-eye observations	High turbidity on complexation Change in the solution color	Malay et al. (2007)
Silk fibroin-cellulose	Cellulose fiber with SF powder	Wrinkle-recovery angles measurements with wrinkle-recovery tester	Crease resistance property increased	Lin et al. (2008)
Silk fibroin-chitosan	Particle	Viscosity measurements by viscometer Light scattering measurement with Electrophoretic Light Scattering Spectrophotometer Thermal behavior analysis with DSC 2910	Decrease of the viscosity of the mixed solution Narrow range of particles sizes Higher thermal decomposition temperature (T_d)	Kang et al. (2004)
Silk fibroin-chitosan	Particle	Amino acid composition by HPLC	Decreased Tyr concentration	Sampaio et al. (2005)

[a] Authors added.

5.6. Influence of interactions on swelling properties

Swelling properties of films or hydrogels in aqueous solutions are very important in biomaterials applications. Du et al. (2006) found that the swelling ratios of silk fibroin-chitosan blending films increased with more chitosan content. It can be inferred that swelling ratios were related to the hydrophilic groups content and the intermolecular interactions in the blending system. When silk fibroin and chitosan were blended, some intermolecular interactions might occur, and possibly some complicated intermolecular structure might form in the blending films, such as an interpenetrating polymer network (IPN). He et al. (2010) stated that swelling capacity reflected the water retaining capacity of a membrane, and their results showed the blending system with 5–10% of carboxymethyl chitosan (CMCS) had lower swelling capacity, which may be affected by their degree of hydrogen bonding as well as crystallinity.

5.7. Influence of interactions on turbidity

In order to investigate the intermolecular interactions in solutions, turbidity is an effective assessing factor. Malay et al. (2007) studied silk fibroin-hyaluronic acid blending solutions and found that turbidimetric curves of the acid titrated silk fibroin–hyaluronic acid mixtures. The two figures revealed the complex formation between silk fibroin and hyaluronic acid favored the pH range (2.5–3.9). When pH > $IEP_{silk\ fibroin}$, both silk fibroin and hyaluronic acid were negatively charged and the complexation did not occur. While pH was lower than $IEP_{silk\ fibroin}$, the value of system turbidity increased rapidly, perhaps due to the aggregation of many

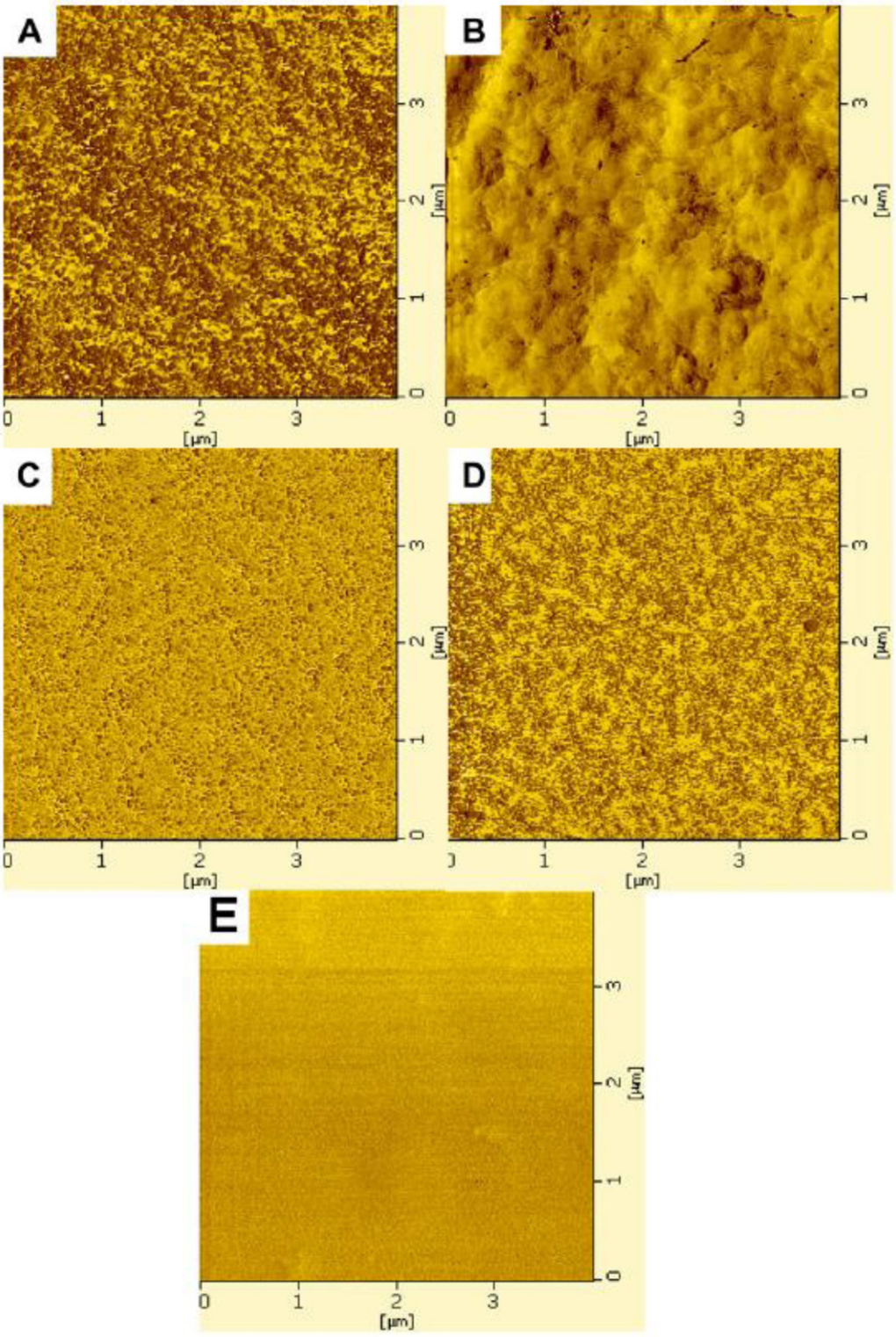

Fig. 12. AFM phase images of the silk fibroin-cellulose blend films.

inter- and intra-polymeric complexes, which was beneficial to the formation of electrostatic complexes.

6. Future perspectives

A future challenge is to create polysaccharide and protein composites or blends with tailored structures and properties according to various interaction modes for wide applications. Furthermore, besides IR analysis for investigating intermolecular interactions between natural polysaccharides and silk fibroin protein, Raman and NMR analysis will be applied increasingly. In addition, with the advance of the co-solvents for natural polysaccharides and silk fibroin protein, such as ionic liquids, their composites or blends will increase greatly in different applications and deeper understanding of intermolecular interactions will be developed.

7. Conclusions

Natural polysaccharides can mainly form hydrogen bonds, electrostatic interactions and covalent bonds with silk fibroin protein. In addition, cross-linkers can bridge them with improved properties. Natural cross-linkers, in particular, have attracted much attention and are promising due to their similar cross-linking ability as chemical cross-linkers, together with reduced chemical toxicity and cytotoxicity. The conformation transition of silk fibroin protein caused by interactions was detected by IR analysis. The presence of interactions actually improved the properties of the polymer matrix, such as crease resistance, mechanical, thermal, miscibility, water stability and swelling properties. A challenge for future studies is to create blends or composites with a tailored structure and properties according to the various interaction modes.

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